

APPENDIX A: PENDING CLAIMS

1 1. A method for modifying the glycosylation pattern of a glycopeptide
2 comprising an acceptor moiety for a first fucosyltransferase wherein said fucosyltransferase
3 lacks a membrane anchoring domain, said method comprising:
4 contacting the glycopeptide with a reaction mixture that comprises a fucose donor
5 moiety and the first fucosyltransferase under appropriate conditions to transfer fucose from the
6 fucose donor moiety to the acceptor moiety, such that the glycopeptide has a substantially
7 uniform fucosylation pattern.

1 2. The method according to claim 1, wherein the glycopeptide comprises a
2 second acceptor moiety for a second fucosyltransferase, and the method further comprises
3 contacting the glycopeptide with a reaction mixture that comprises a fucose donor moiety and the
4 second fucosyltransferase under appropriate conditions to transfer fucose from the fucose donor
5 moiety to the acceptor moiety, such that the glycopeptide has a substantially uniform
6 fucosylation pattern.

1 3. The method according to claim 2, wherein the glycoprotein is contacted
2 with the first fucosyltransferase and the second fucosyltransferase simultaneously.

1 4. The method according to claim 2, wherein the glycoprotein is contacted
2 with the first fucosyltransferase and the second fucosyltransferase sequentially without isolation
3 of product resulting from contacting with the first fucosyltransferase.

1 5. The method according to claim 1, wherein the first fucosyltransferase is a
2 member selected from FucT-IV, FucT-VI, FucT-VII and combinations thereof.

1 6. The method according to claim 2, wherein the second fucosyltransferase is
2 a member selected from FucT-IV, FucT-VI, FucT-VII and combinations thereof.

1 7. The method of claim 1, wherein the fucosyltransferase is bacterial.

- 1 8. The method of claim 1, wherein the fucosyltransferase is recombinantly
2 produced.
- 1 9. The method of claim 1, wherein the fucosyltransferase lacks a membrane
2 anchoring domain.
- 1 10. The method of claim 1, wherein at least about 80% of the acceptor
2 moieties on the glycopeptide are fucosylated.
- 1 11. The method of claim 1, wherein glycopeptide is reversibly immobilized on
2 a solid support.
- 1 12. The method of claim 1, wherein the solid support is an affinity
2 chromatography medium.
- 1 13. The method of claim 1, wherein the glycopeptide is a full-length
2 glycopeptide.
- 1 14. The method of claim 1, wherein the glycopeptide is a fragment of a full
2 length glycopeptide comprising an active site of the full-length glycopeptide.
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- 1 15. The method according claim 1, wherein the glycopeptide is an IgG
2 chimera.
- 1 16. The method of claim 1, wherein the glycopeptide is a hormone, a growth
2 factor, an enzyme, an enzyme inhibitor, a cytokine, a receptor, a ligand, or a monoclonal
3 antibody.
- 1 17. The method of claim 1, wherein the glycopeptide is on a cell.
- 1 18. The method of claim 1, wherein the acceptor moiety comprises Gal β 1-OR,
2 Gal β 1,3/4GlcNAc-OR, NeuAc α 2,3Gal β 1,3/4GlcNAc-OR, wherein R is an amino acid, a

3 saccharide, an oligosaccharide or an aglycon group having at least one carbon atom and is linked
4 to or is part of a glycopeptide.

1 19. The method of claim 1, wherein the fucose donor moiety is GDP-fucose.

1 20. The method of claim 1, further comprising, prior to step (a), contacting
2 said glycoprotein with a glycosyltransferase other than a fucosyltransferase and a donor moiety
3 other than a fucose donor moiety, thereby glycosylating the glycoprotein with a glycosyl moiety
4 other than a fucose unit.

1 21. The method of claim 20, wherein the glycosyltransferase is a member
2 selected from the group consisting of galactosyltransferase, sialyltransferase and combinations
3 thereof.

1 31. A method of producing a recombinant glycopeptide having a fucosylation
2 pattern that is substantially identical to a fucosylated glycopeptide having a known fucosylation
3 pattern, said method comprising:

4 (a) contacting the recombinant glycopeptide with a reaction mixture that comprises a
5 fucose donor moiety and the fucosyltransferase under appropriate conditions to
6 transfer fucose from the fucose donor moiety to a fucose acceptor moiety on said
7 recombinant glycopeptide, thereby producing a fucosylated recombinant
8 glycopeptide, wherein said fucosyltransferase lacks a membrane anchoring
9 domain ; and

10 (b) terminating the transfer of the fucose to the fucose acceptor when the fucosylation
11 pattern substantially identical to the known fucosylation pattern is obtained.

1 32. The method according to claim 31 further comprising:

2 (c) assaying the fucosylation pattern of the fucosylated recombinant glycopeptide,
3 thereby determining whether the fucosylation pattern is substantially identical to
4 the known fucosylation pattern.

1 33. The method according to claim 31 wherein the terminating is due to
2 exhausting in the reaction mixture a member selected from the group consisting of the
3 fucosyltransferase, the fucose donor moiety, the fucose acceptor quench with a chelator and
4 combinations thereof.

1 34. The method according to claim 31, wherein the glycopeptide comprises a
2 second acceptor moiety for a second fucosyltransferase, and the method further comprises
3 contacting the glycopeptide with a reaction mixture that comprises a fucose donor moiety and the
4 second fucosyltransferase under appropriate conditions to transfer fucose from the fucose donor
5 moiety to the second acceptor moiety.

1 35. The method according to claim 34, wherein the glycoprotein is contacted
2 with the first fucosyltransferase and the second fucosyltransferase simultaneously.

1 36. The method according to claim 34, wherein the glycoprotein is contacted
2 with the first fucosyltransferase and the second fucosyltransferase sequentially without isolation
3 of product resulting from contacting with the first fucosyltransferase.

1 37. The method according to claim 31, wherein the first fucosyltransferase is a
2 member selected from FucT-IV, FucT-VI, FucT-VII and combinations thereof.

1 38. The method according to claim 34, wherein the second fucosyltransferase
2 is a member selected from FucT-IV, FucT-VI, FucT-VII and combinations thereof.

1 39. The method of claim 31, wherein the fucosyltransferase is bacterial.

1 40. The method of claim 31, wherein the fucosyltransferase is recombinantly
2 produced.

1 41. The method of claim 31, wherein the fucosyltransferase lacks a membrane
2 anchoring domain.

1 42. The method of claim 31, wherein at least about 80% of the acceptor
2 moieties on the glycopeptide are fucosylated.

1 43. The method of claim 31, wherein glycopeptide is reversibly immobilized
2 on a solid support.

1 44. The method of claim 31, wherein the solid support is an affinity
2 chromatography medium.

1 45. The method of claim 31, wherein the glycopeptide is a full-length
2 glycopeptide.

1 46. The method of claim 31, wherein the glycopeptide is a fragment of a full
2 length glycopeptide comprising an active site of the full-length glycopeptide.

1 47. The method according claim 31, wherein the glycopeptide is an IgG
2 chimera.

1 48. The method of claim 31, wherein the glycopeptide is a hormone, a growth
2 factor, an enzyme, an enzyme inhibitor, a cytokine, a receptor, a ligand, or a monoclonal
3 antibody.

1 49. The method of claim 31 wherein the glycopeptide is on a cell.

1 50. The method of claim 31, wherein the acceptor moiety comprises Gal β 1-
2 OR, Gal β 1,3/4GlcNAc-OR, NeuAc α 2,3Gal β 1,3/4GlcNAc-OR, wherein R is an amino acid, a
3 saccharide, an oligosaccharide or an aglycon group having at least one carbon atom and is linked
4 to or is part of a glycopeptide.

1 51. The method of claim 31, wherein the fucose donor moiety is GDP-fucose.

1 **52.** The method of claim 31, further comprising, prior to step (a), contacting
2 said glycoprotein with a glycosyltransferase other than a fucosyltransferase and a donor moiety
3 other than a fucose donor moiety, thereby glycosylating the glycoprotein with a glycosyl moiety
4 other than a fucose unit.

1 **53.** The method of claim 52, wherein the glycosyltransferase is a member
2 selected from the group consisting of galactosyltransferase, sialyltransferase and combinations
3 thereof.

1 **54.** A large-scale method for modifying the glycosylation pattern of a
2 glycopeptide comprising an acceptor moiety for a first fucosyltransferase, said method
3 comprising:
4 contacting at least about 500 mg of glycopeptide with a reaction mixture that
5 comprises a fucose donor moiety and the first fucosyltransferase under appropriate conditions to
6 transfer fucose from the fucose donor moiety to the acceptor moiety, such that the glycopeptide
7 has a substantially uniform fucosylation pattern.

1 **55.** A large-scale method of producing a recombinant glycopeptide having a
2 fucosylation pattern that is substantially identical to a fucosylated glycopeptide having a known
3 fucosylation pattern, said method comprising:

4 (a) contacting at least about 500 mg of the recombinant glycopeptide with a
5 reaction mixture that comprises a fucose donor moiety and the fucosyltransferase under
6 appropriate conditions to transfer fucose from the fucose donor moiety to a fucose acceptor
7 moiety on said recombinant glycopeptide, thereby producing a fucosylated recombinant
8 glycopeptide; and

9 (b) terminating the transfer of the fucose to the fucose acceptor when the
10 fucosylation pattern substantially identical to the known fucosylation pattern is obtained.

1 **56.** A method for modifying the glycosylation pattern of a glycopeptide
2 comprising a first acceptor moiety for a first fucosyltransferase and a second acceptor moiety for
3 a second fucosyltransferase, said method comprising:

- 4 (a) contacting the glycopeptide with a reaction mixture that comprises a fucose donor
5 moiety and the first fucosyltransferase under appropriate conditions to transfer
6 fucose from the fucose donor moiety to the first acceptor moiety, such that the
7 glycopeptide has a substantially uniform fucosylation pattern, and
8 (b) contacting the glycopeptide with a reaction mixture that comprises a fucose donor
9 moiety and the second fucosyltransferase under appropriate conditions to transfer
10 fucose from the fucose donor moiety to the second acceptor moiety, such that the
11 glycopeptide has a substantially uniform fucosylation pattern.

1 57. A method for modifying the glycosylation pattern of a glycopeptide
2 comprising a first acceptor moiety for a first fucosyltransferase and a second acceptor moiety for
3 a second fucosyltransferase, wherein the glycopeptide is contacted with the first
4 fucosyltransferase and the second fucosyltransferase simultaneously, said method comprising:

- 5 (a) contacting the glycopeptide with a reaction mixture that comprises a fucose donor
6 moiety and the first fucosyltransferase under appropriate conditions to transfer
7 fucose from the fucose donor moiety to the first acceptor moiety, such that the
8 glycopeptide has a substantially uniform fucosylation pattern, and
9 (b) contacting the glycopeptide with a reaction mixture that comprises a fucose donor
10 moiety and the second fucosyltransferase under appropriate conditions to transfer
11 fucose from the fucose donor moiety to the second acceptor moiety, such that the
12 glycopeptide has a substantially uniform fucosylation pattern.

1 58. A method for modifying the glycosylation pattern of a glycopeptide
2 comprising a first acceptor moiety for a first fucosyltransferase and a second acceptor moiety for
3 a second fucosyltransferase, wherein the glycopeptide is contacted with the first
4 fucosyltransferase and the second fucosyltransferase sequentially without isolation of product
5 resulting from contacting with the first fucosyltransferase, said method comprising:

- 6 (a) contacting the glycopeptide with a reaction mixture that comprises a fucose donor
7 moiety and the first fucosyltransferase under appropriate conditions to transfer
8 fucose from the fucose donor moiety to the first acceptor moiety, such that the
9 glycopeptide has a substantially uniform fucosylation pattern, and

10 (b) contacting the glycopeptide with a reaction mixture that comprises a fucose donor
11 moiety and the second fucosyltransferase under appropriate conditions to transfer
12 fucose from the fucose donor moiety to the second acceptor moiety, such that the
13 glycopeptide has a substantially uniform fucosylation pattern.

1 59. A method for modifying the glycosylation pattern of a glycopeptide
2 comprising an acceptor moiety for a first fucosyltransferase, wherein the first fucosyltransferase
3 is a member selected from FucT-IV, FucT-VI, FucT-VII and combinations thereof, said method
4 comprising:

5 contacting the glycopeptide with a reaction mixture that comprises a fucose donor moiety
6 and the first fucosyltransferase under appropriate conditions to transfer fucose
7 from the fucose donor moiety to the acceptor moiety, such that the glycopeptide
8 has a substantially uniform fucosylation pattern.

1 60. A method for modifying the glycosylation pattern of a glycopeptide
2 comprising a first acceptor moiety for a first fucosyltransferase and a second acceptor moiety for
3 a second fucosyltransferase, wherein the second fucosyltransferase is a member selected from
4 FucT-IV, FucT-VI, FucT-VII and combinations thereof, said method comprising:

5 (a) contacting the glycopeptide with a reaction mixture that comprises a fucose donor
6 moiety and the first fucosyltransferase under appropriate conditions to transfer
7 fucose from the fucose donor moiety to the first acceptor moiety, such that the
8 glycopeptide has a substantially uniform fucosylation pattern, and

9 (b) contacting the glycopeptide with a reaction mixture that comprises a fucose donor
10 moiety and the second fucosyltransferase under appropriate conditions to transfer
11 fucose from the fucose donor moiety to the second acceptor moiety, such that the
12 glycopeptide has a substantially uniform fucosylation pattern.

1 61. A method for modifying the glycosylation pattern of a glycopeptide
2 comprising an acceptor moiety for a first fucosyltransferase, wherein the first fucosyltransferase
3 is recombinantly produced, said method comprising:

4 contacting the glycopeptide with a reaction mixture that comprises a fucose donor moiety
5 and the first fucosyltransferase under appropriate conditions to transfer fucose
6 from the fucose donor moiety to the acceptor moiety, such that the glycopeptide
7 has a substantially uniform fucosylation pattern.

1 62. The method according to claim 61, wherein said first fucosyltransferase
2 lacks a membrane anchoring domain.

1 63. A method for modifying the glycosylation pattern of a glycopeptide
2 comprising an acceptor moiety for a first fucosyltransferase wherein the glycopeptide is an IgG
3 chimera, said method comprising:
4 contacting the glycopeptide with a reaction mixture that comprises a fucose donor
5 moiety and the first fucosyltransferase under appropriate conditions to transfer fucose from the
6 fucose donor moiety to the acceptor moiety, such that the glycopeptide has a substantially
7 uniform fucosylation pattern.

1 64. A method for modifying the glycosylation pattern of a glycopeptide
2 comprising an acceptor moiety for a first fucosyltransferase, said method comprising:
3 (a) contacting said glycoprotein with a glycosyltransferase other than a fucosyltransferase
4 and a donor moiety other than a fucose donor moiety, thereby glycosylating the
5 glycoprotein with a glycosyl moiety other than a fucose unit, and
6 (b) contacting the glycopeptide with a reaction mixture that comprises a fucose donor
7 moiety and the first fucosyltransferase under appropriate conditions to transfer
8 fucose from the fucose donor moiety to the acceptor moiety, such that the
9 glycopeptide has a substantially uniform fucosylation pattern.

1 65. A method for modifying the glycosylation pattern of a glycopeptide
2 comprising an acceptor moiety for a first fucosyltransferase, said method comprising:
3 (a) contacting said glycoprotein with a glycosyltransferase other than a fucosyltransferase
4 and a donor moiety other than a fucose donor moiety, thereby glycosylating the
5 glycoprotein with a glycosyl moiety other than a fucose unit, wherein the

6 glycosyltransferase is a member selected from the group consisting of
7 galactosyltransferase, sialyltransferase and combinations thereof, and
8 (b) contacting the glycopeptide with a reaction mixture that comprises a fucose donor
9 moiety and the first fucosyltransferase under appropriate conditions to transfer
10 fucose from the fucose donor moiety to the acceptor moiety, such that the
11 glycopeptide has a substantially uniform fucosylation pattern.